

## Diagnosis of Early Lyme Disease by Polymerase Chain Reaction Amplification and Culture of Skin Biopsies from Erythema Migrans Lesions

IRA SCHWARTZ,<sup>1\*</sup> GARY P. WORMSER,<sup>2</sup> JOHN J. SCHWARTZ,<sup>1</sup> DENISE COOPER,<sup>2</sup>  
PAUL WEISSENSEE,<sup>1</sup> ANNA GAZUMYAN,<sup>1</sup> ELLEN ZIMMERMANN,<sup>1</sup> NEIL S. GOLDBERG,<sup>3</sup>  
SUSAN BITTKER,<sup>2</sup> GRANT L. CAMPBELL,<sup>4</sup> AND CHARLES S. PAVIA<sup>2</sup>

Departments of Biochemistry and Molecular Biology,<sup>1</sup> Medicine,<sup>2</sup> and Dermatology,<sup>3</sup> New York Medical College, Valhalla, New York 10595, and Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522<sup>4</sup>

Received 18 May 1992/Accepted 27 August 1992

Current laboratory diagnosis of Lyme disease relies on tests for the detection of antibodies to *Borrelia burgdorferi*, the etiologic agent of the disease. These tests are often unreliable because of a lack of sensitivity and specificity and test-to-test variability. The purpose of this study was to evaluate the sensitivity and specificity of polymerase chain reaction (PCR) amplification for detection of *B. burgdorferi* in skin biopsy specimens. Forty-six 2-mm skin biopsy samples were obtained from 44 patients with a clinical diagnosis of erythema migrans, 9 of whom were receiving antibiotic therapy at the time of biopsy. Specimens were ground in BSK medium with separate aliquots taken for culture and PCR. Of the specimens from the untreated group, 57% (21 of 37) were positive by culture and 22% (8 of 37) were culture negative; 22% (8 of 37) of the cultures were uninformative because of contamination. By comparison, 22 (59%) of 37 specimens were positive by PCR amplification. Of 21 culture-positive samples, 13 (62%) were also positive by PCR analysis. Thus, the sensitivity of the PCR was 59 to 62%, based on either a clinical or cultural diagnosis of untreated Lyme disease. None of the nine specimens from antibiotic-treated patients grew in culture, whereas two of the nine were positive by PCR analysis. Given the complexity and time required for culture, PCR is a promising technique for the diagnosis of early Lyme disease.

Lyme disease is the most commonly reported vector-borne disease in the United States (35). The disease is frequently diagnosed on the basis of the presence of the characteristic skin lesion, erythema migrans (EM), which often occurs in conjunction with mild constitutional symptoms (27, 35, 37). Current laboratory diagnosis consists of a variety of serological assays for detection of antibodies to antigens of *Borrelia burgdorferi*, the etiologic agent (2, 36). These diagnostic tests suffer from a number of limitations, including lack of sensitivity (false-negativity) and specificity (false-positivity) and significant intra- and interlaboratory variability (8, 17, 31, 38).

A further shortcoming of serological testing is that it is indirect, i.e., it does not recognize the presence of the spirochete itself but rather the infected host's response. As such, the tests do not necessarily indicate active infection, only the occurrence of infection at some undefined time. The diagnosis of most bacterial diseases relies on culture of the etiologic agent. Recovery of *B. burgdorferi* from blood (2, 20, 28, 36), cerebrospinal fluid (14, 28, 36), synovial fluid (30, 33), skin (4, 5, 21, 28, 36), and cardiac tissue (34) has been reported. However, the yield is generally below 10% from sites other than skin, and many weeks of incubation may be required before growth can be detected. These factors reduce the clinical utility of culture as a routine diagnostic modality for Lyme disease.

Furthermore, clinical uncertainty may arise when the rash is atypical or resembles other cutaneous entities or when concomitant constitutional symptoms are absent (3). Thus,

at present there is no completely satisfactory bedside or laboratory test to confirm the diagnosis of Lyme disease in patients who present with a skin lesion or other manifestations of early disease.

The polymerase chain reaction (PCR) has come into increasing use for detecting infectious agents that may be difficult or impossible to grow in culture (10, 26). The method has been employed for detection of *B. burgdorferi* in vitro, in spirochete-seeded serum, and in ticks (18, 24, 25, 29), and several reports of its application to human clinical specimens have appeared (11-13, 15, 19). The potential sensitivity, specificity, and rapid return of results of the PCR make it a particularly appealing procedure for detecting *B. burgdorferi*.

Previous demonstrations of viable *B. burgdorferi* in EM lesions and the relative accessibility of such tissue suggested that skin biopsy samples obtained from EM lesions would be excellent material for PCR-based detection of *B. burgdorferi* in early Lyme disease. To assess the utility of the PCR, a study of a series of patients with a clinical diagnosis of EM was undertaken. Skin biopsies of EM lesions from these patients were subjected to both the PCR and culture. The results indicate that the PCR is a promising technique for the diagnosis of early Lyme disease.

### MATERIALS AND METHODS

**Skin biopsy and culture.** The research protocol described here was approved by the Internal Review Board of New York Medical College. After the skin surface was disinfected and local anesthesia was induced with 1% lidocaine plus 1:100,000 epinephrine, a 2-mm punch biopsy was taken from

\* Corresponding author.

the advancing border of a possible primary EM lesion of patients attending the Lyme Disease Diagnostic Center of the Westchester County Medical Center between July and September 1991. All patients satisfied the Centers for Disease Control surveillance definition for Lyme disease with EM (7). Control skin samples included those from patients with rashes atypical for EM and discarded samples of skin provided from 10 anonymous patients undergoing plastic surgery procedures. No medical history was available for the latter cases.

The biopsy material was immediately placed into a micro-centrifuge tube containing 0.5 ml of BSK medium (1, 20) lacking normal rabbit serum and gelatin (incomplete BSK) for transport to a tissue culture hood. The specimen was removed from the medium and placed into a micro-tissue grinder (Spectrum Brand) consisting of a (10 by 50 mm) reservoir glass tube with a 5-mm sphere pestle. Fresh incomplete BSK (0.4 ml) was added, and the biopsy material was ground. Then 0.1 ml of this suspension was removed for PCR analysis (see below). A separate 0.1-ml sample was added to a 7-ml screw-cap tube containing 6 ml of complete BSK medium. The latter tube was tightly capped and incubated at 33°C. Aliquots (10 µl) of each biopsy culture were examined for the presence of spirochetes as previously described (20) with the fluorescence microscopy technique of Pavia and Niederbuhl (22). All cultures were first examined at 2 weeks and incubated for at least 8 weeks.

**Serology.** Patient sera were tested for the presence of antibodies to *B. burgdorferi* at the first and subsequent visit(s) by using a commercially available enzyme-linked immunosorbent assay system (Stat Lyme ELISA; Whittaker Bioproducts, Walkersville, Md.) designed to measure both immunoglobulins G and M. The enzyme-linked immunosorbent assay was carried out in accordance with the manufacturer's instructions.

**PCR of skin biopsy tissue.** Samples (0.1 ml) of skin biopsy suspensions (see above) were provided as coded specimens. Suspensions prepared from control and test skin biopsies were provided in a random manner. DNA amplification was performed as follows. Samples were centrifuged for 5 to 15 min at 12,000 × *g* to pellet spirochetes. The pellets were washed once, resuspended in 30 µl of buffer (10 mM Tris-HCl [pH 7.4], 0.5% Nonidet P-40, 0.5% Tween 20, 100 µg of proteinase K per ml), incubated for 1 h at 55°C, and then boiled for 15 min. Samples of 15 µl were removed and processed for PCR amplification in 50 µl of a solution containing 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.1% (wt/vol) gelatin, 100 µM (each) dATP, dGTP, dCTP, and TTP, 1.25 U of *Taq* polymerase (Perkin-Elmer/Cetus), and 15 pmol of each primer (Fig. 1). The amplification reaction was carried out for 35 cycles in a DNA thermal cycler (Perkin-Elmer/Cetus) with an amplification profile of denaturation at 94°C for 1 min, annealing at 43°C for 1 min, and extension at 72°C for 1 min. After amplification, the reaction mixtures were applied to nylon membranes, and the amplified product was detected by hybridization to a <sup>32</sup>P-labelled oligonucleotide probe complementary to a sequence contained on the amplified product (Fig. 1) and autoradiography for 12 to 24 hours. For most specimens, amplification was performed a minimum of two times (usually once with each of the two different primer pairs); a sample was not considered positive unless the amplified product was obtained in at least two separate experiments.

Pre- and post-PCR sample processing and the amplification reaction itself were carried out in physically separate

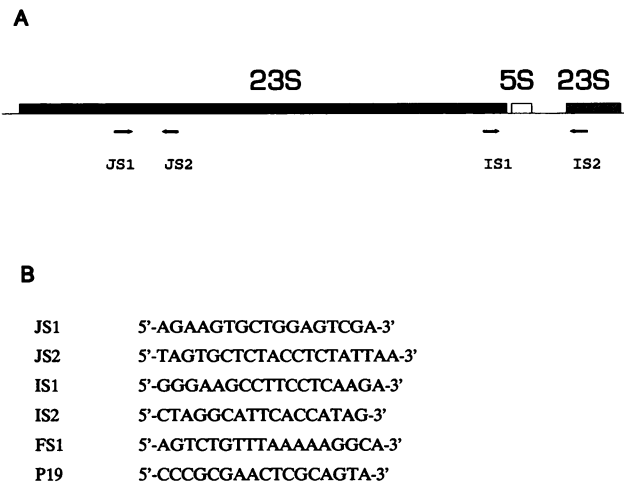


FIG. 1. (A) Schematic diagram of a portion of the rRNA operon of *B. burgdorferi*. The forward and reverse arrows indicate the locations of the PCR primer pairs relative to the 23S rRNA sequence. (B) Sequences of the oligonucleotides employed as primers and probes in this study. FS1 and P19 are probes for JS1-JS2 and IS1-IS2 amplicons, respectively.

rooms to prevent cross-contamination and sample carry-over. In addition, positive-displacement pipetting devices or plugged pipet tips were used to eliminate aerosols.

**Nucleotide sequence accession number.** The sequences for *B. burgdorferi* 16S and 23S rRNA genes have been deposited in GenBank with accession numbers M88329 and M88330, respectively.

**Statistics.** Proportions were compared by using a two-tailed Fisher exact test. A *P* value of ≤0.05 was considered significant.

## RESULTS

**Culture of *B. burgdorferi* from skin biopsy specimens.** The study group included 44 patients thought to have EM on the basis of clinical presentation. Only a single lesion per patient was biopsied except for one patient, from whom biopsy samples of three separate lesions were taken. Nine patients had received at least one dose of an antibiotic at the time of skin biopsy, and the remaining 35 were untreated. Fifty-seven percent (21 of 37) of the skin biopsy specimens from this latter group were culture positive for *B. burgdorferi*. Twenty-two percent (8 of 37) were culture negative and 22% (8 of 37) of the cultures were inconclusive because of contamination (Table 1). Excluding the eight instances in which contamination occurred, the yield of *B. burgdorferi* from culture was 72% (21 of 29). All *B. burgdorferi* isolates from the initial culture of the skin biopsies were subcultured and maintained through several passages. In contrast, *B. burgdorferi* could not be recovered from any of the nine patients who had received prior antibiotic treatment despite incubation for an average of 11 weeks (range, 9 to 13 weeks).

All evaluable (i.e., noncontaminated) control specimens were culture negative. These included skin samples from 10 individuals undergoing plastic surgery procedures and from two of three patients (one was contaminated) with atypical rashes of uncertain etiology (all three patients tested seronegative against *B. burgdorferi* on presentation). Biopsies were performed again on 13 of the culture-positive patients after resolution of the EM at a site adjacent to the original

TABLE 1. Results of antibody testing, culture, and PCR on patients with a clinical diagnosis of EM

History and patient no.	Test results			
	Culture	Serology		PCR
		Acute phase	Convalescent phase	
No prior antibiotic therapy				
1	+	+	+	(21) <sup>a</sup> +
2	—	+	+	(25) +
3	+	+	+	(28) +
4	+	+	+	(35) +
5	+	—	+	(31) +
6	+	+	+	(29) +
7	+	—	—	(33) +
8	+	+	+	(33) +
9	+	—	+	(29) +
10	+	—	+	(26) +
11	Contaminated	—	NA <sup>b</sup>	—
12	+	—	+	(26) +
13	+	—	+	(24) —
14	+	+	+	(30) —
15	+	—	+	(29) —
16	Contaminated	—	—	(29) +
17	—	+	NA	—
18	+	+	+	(28) +
19	—	—	—	(29) —
20	Contaminated	—	—	(28) +
21 <sup>c</sup>	—, +, Contaminated	+	+	(28) +, +, +
22	+	—	+	(30) —
23	Contaminated	+	+	(29) +
24	+	—	+	(21) +
25	+	—	+	(27) —
26	—	—	+	(24) —
27	—	+	+	(21) +
28	Contaminated	—	—	(26) +
29	—	—	—	(29) —
30	Contaminated	+	+	(28) —
31	+	—	+	(35) —
32	+	+	+	(31) —
33	Contaminated	—	NA	—
34	+ <sup>d</sup>	—	+	(29) +
35	+	+	+	(24) —
Prior antibiotic therapy <sup>e</sup>				
1	—	+	+	(21) +
2	—	—	+	(55) +
3	—	—	+	(30) —
4	—	—	—	(35) —
5	—	+	+	(43) —
6	—	+	+	(23) —
7	—	—	+	(23) —
8	—	+	+	(31) —
9	—	—	—	(32) —

<sup>a</sup> Numbers within parentheses indicate the number of days from the initial serological test.

<sup>b</sup> NA, not available.

<sup>c</sup> Three separate lesions from this patient were biopsied.

<sup>d</sup> Culture was negative in BSK but positive in another medium.

<sup>e</sup> Antibiotic therapy had already been received at the time of skin biopsy as follows (patient numbers): 1, one dose of amoxicillin (500 mg); 2, five doses of tetracycline (250 mg); 3, one dose of amoxicillin (500 mg); 4, two doses of doxycycline (100 mg); 5, spiramycin (4 days, dose not available); 6, one dose of doxycycline (100 mg); 7, six doses of doxycycline (100 mg); 8, one dose of doxycycline (100 mg); 9, one dose of tetracycline (500 mg).

biopsy site. Repeat biopsies were performed within 1 month after completion of antibiotic therapy (except for one case, for whom it was carried out several months later). Four of the cultures of these biopsies were contaminated, but the remainder were culture negative after at least 13 weeks of incubation.

**rRNA-based PCR detection assay for *B. burgdorferi*.** The organization of the rRNA genes of *B. burgdorferi* and the sequences of the corresponding rRNAs have been determined (32). Figure 1 presents a schematic diagram of the rRNA operon and the positions of the primers and probes employed for PCR amplification and detection. The 23S rRNA sequence was compared for homology to other rRNA sequences in the GenBank data base. On the basis of these comparisons, a region near the 5' end of the 23S RNA sequence (nucleotides 689 through 948) was chosen as a likely target for amplification. The equivalent regions of the 23S rRNA genes in the related species *Borrelia hermsii* and *B. anserina* and several isolates of *B. burgdorferi* were also sequenced (Fig. 2). PCR primers (designated JS1 and JS2) were designed to contain perfect homology to the *B. burgdorferi* sequence but maximum mismatch at their 3' ends with the related *Borrelia* species (Fig. 2). The sensitivity of the PCR assay was determined with serially diluted, titrated *B. burgdorferi* samples. Fewer than 10 spirochetes in a total sample could be detected efficiently (Fig. 3). The sensitivity and specificity of the assay were also investigated by performing PCR amplification with 10 different isolates of *B. burgdorferi*, *B. hermsii*, *B. anserina*, and *Borrelia turicatae*. Samples containing 50 spirochetes were subjected to PCR amplification, and one-fifth of the amplified product (equal to 10 spirochetes) was detected by hybridization with a radio-labeled probe (FS1) corresponding to a portion of the amplified sequence. All isolates of *B. burgdorferi* were detected by the procedure with essentially equal efficiency (Fig. 4). These included isolates from North America (isolates 24430, 24352, HK, B31, 297), Europe (20004, G1, 20047), and Russia (IP90, IP3). Furthermore, only *B. burgdorferi* was detected by this method; samples containing the other closely related *Borrelia* species produced no amplified product.

To provide a second primer pair that could be employed for specific detection of *B. burgdorferi*, we took advantage of the unusual and unique tandem duplication of the 23S rRNA gene (Fig. 1). This feature was observed in all *B. burgdorferi* isolates tested and, furthermore, was not found in other *Borrelia* species (32). Thus, a PCR amplimer pair with the forward primer targeted to a sequence at the 3' end of the first copy of 23S RNA gene and a reverse primer complementary to a sequence near the 5' end of the second 23S RNA gene copy should have absolute specificity for *B. burgdorferi*. The locations of this primer pair (designated IS1 and IS2, respectively) relative to the rRNA operon are presented in Fig. 1. The sensitivity and specificity of this primer pair were tested in a manner similar to that described above for the JS1-JS2 primer pair. The IS1-IS2 amplimer set displayed a degree of specificity and sensitivity similar to that of JS1-JS2 (Fig. 5).

**Detection of *B. burgdorferi* in skin biopsy specimens by the PCR.** Of 37 samples obtained from the 35 patients who had not received antibiotics, 22 (59%) yielded a positive signal for the presence of *B. burgdorferi*. Of the 21 culture-positive specimens, 13 (62%) were positive by the PCR. *B. burgdorferi* was also detected by the PCR in 50% (4 of 8) of the culture-negative samples and 63% (5 of 8) of the culture-contaminated samples (not statistically significant for each

BB	ATTAAAGCAT	<b>AGAAGTCTG</b>	<b>GAGTCGAAGC</b>	GAAAGGGTTC	TTAAAAGGGC	GATTAGTTA	739
BH			C TG		C		
BA			C TG		C		
BB	GATGTGGTAG	ACCCGAAGCC	GAGTGATCTA	TTTATGGCCA	GGCTGAAGCT	TGGGTAAAC	799
BH		A					
BA							
BB	CAAGTGGAGG	GCCGAAGTCT	AGTCTGTTTA	AAAAGGCAGG	GATGAGCTGT	GAATAGGAGT	859
BH		C					
BA							
BB	GAAAGGCTAA	ACAAACTCGG	AGATAGCTGG	TTCTCCCCGA	AATGGATTTA	AGTTCAGCCT	919
BH				T			
BA				T			
BB	TATTTTAGTT	<b>TAATAGAGGT</b>	<b>AGAGCACTAA</b>	TTGAGCTAGG	GCCTGTCAAA	GGGTACCAA	979
BH		G C					
BA		G C					
BB	CTCAGTTAAA	CTCCGAATGC	TATTAATGA	TGAATAGGAG	TGAGACTATG	GGCGATAAGG	1039
BH			GT			T	
BA			GC				
BB	TTCATAGTCG	AGAGGGAAAC	AACCCAGACC	AACAGCTAAG	GTCTCAAAAA	TGTGTTAAGT	1099
BH	C	G					
BA		G					
BB	GGAAAAGGAG	GTTTAGGTAC	GTAACAGCC	AGGAGGTTGG	CTTAGAAGCA	GCCA	1153
BH							
BA							

FIG. 2. DNA sequence of a portion of the *B. burgdorferi* 23S rRNA gene. The sequence shown is nucleotides 678 through 1153 of the mature 23S rRNA sequence. The sequence differences between *B. burgdorferi* and *B. hermsii* (BH) or *B. anserina* (BA) are indicated below the *B. burgdorferi* sequence. The sequences employed as forward and reverse PCR primers are indicated in boldface type and by forward and reverse arrows, respectively.

comparison). Thus, both culture and the PCR produced approximately equal numbers of *B. burgdorferi*-positive findings in nontreated patient samples (57 and 59%, respectively). Therefore, the sensitivity of the PCR was 59 to 62% based on either a clinical or cultural diagnosis of Lyme disease. In total, 81% (30 of 37) of the samples produced a *B. burgdorferi*-positive result by either culture or the PCR (Table 1). In addition, of the nine samples obtained from patients who had received at least one dose of an antibiotic, two (22%) were positive by the PCR.

Of the 10 control skin samples from patients that were presumed not to have Lyme disease, 1 produced a positive response by the PCR for a specificity of 90%. In addition,

none of the 3 specimens from the group with rashes of uncertain etiology or the 13 specimens from those undergoing repeat biopsy was positive by the PCR. It is reasonable to assume that these latter samples represent negative controls. In that case, the specificity of the PCR test was 96% (25 of 26).

Comparison of the results of the PCR for the evaluable

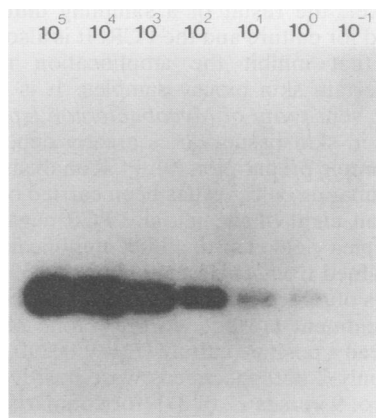


FIG. 3. Sensitivity of PCR amplification with JS1-JS2. The indicated numbers of *B. burgdorferi* cells were lysed and subjected to PCR amplification with the JS1-JS2 amplicon pair. The PCR products were electrophoresed on a 1.5% agarose gel, blotted to nitrocellulose, hybridized to an internal probe, exposed to XAR-5 film for 16 to 18 h, and processed by autoradiography.

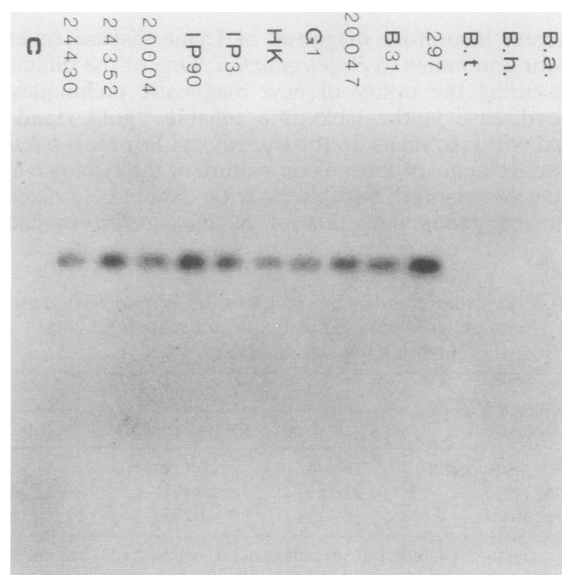


FIG. 4. Specificity of PCR amplification with JS1-JS2. The indicated isolates of *B. burgdorferi* and *B. turicatae* (B.t.), *B. hermsii* (B.h.), and *B. anserina* (B.a.) were amplified by the PCR as described in Materials and Methods. The PCR product from the equivalent of 10 spirochetes was electrophoresed in each case. C is a negative control with no added cells.

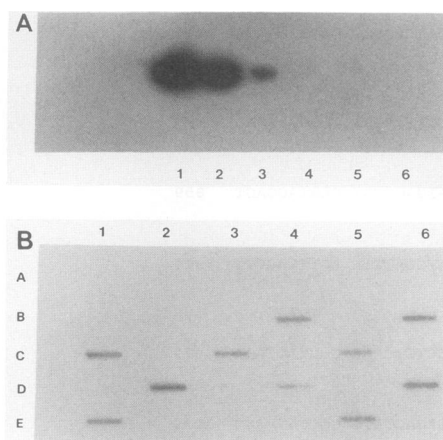


FIG. 5. Sensitivity and specificity of PCR amplification with IS1-IS2. (A) Known amounts of *B. burgdorferi* cells were lysed and analyzed by the PCR with the IS1-IS2 primer pair as described in Materials and Methods and the legend to Fig. 3. Products in lanes 1 through 6 were obtained with  $10^5$ ,  $10^4$ ,  $10^3$ ,  $10^2$ ,  $10^1$ , and  $10^0$  *B. burgdorferi* cells, respectively. (B) PCR amplification with IS1-IS2 primers was carried out with various *B. burgdorferi* isolates as described in Materials and Methods, and the products were examined by slot blot analysis (50 organisms per slot). Shown is an autoradiogram after an overnight exposure. Slots: A1, master mix control; A3, *B. anserina*; A5, *B. hermsii*; B2, *B. turicatae*. The remaining slots contained different isolates of *B. burgdorferi* as follows: B4, B31; B6, 297; C1, IP21; C3, G1; C5, 20047; D2, IP3; D4, 24352; D6, 20004; E1, HK; E5, 24430.

untreated patients who were either initially seropositive, seroconverters, or seronegative on two test dates showed no significant differences. There was also no significant difference in the yield of the culture (Table 2).

## DISCUSSION

Current laboratory diagnosis of Lyme disease relies on tests for antibodies to *B. burgdorferi*. One of the difficulties in assessing the utility of new diagnostic techniques for Lyme disease is the lack of a reliable "gold standard" against which to measure the sensitivity. For most bacterial diseases, diagnosis depends on culture of the etiologic agent as the gold standard, but this criterion cannot be utilized for the unambiguous detection of *B. burgdorferi* in clinical

samples because of the low yield. Since the highest degree of success for culture has been achieved with skin biopsies of EM lesions, this was the clinical material chosen for evaluation of the sensitivity of PCR amplification for *B. burgdorferi*.

Successful culture of *B. burgdorferi* from EM lesions has been reported (4, 5, 16, 21, 28, 36), but usually the yields are much lower than those presented here. In general, cultures were positive only after incubation for several weeks and in extreme cases required up to 10 months (16). Recently, Berger et al. reported the isolation of *B. burgdorferi* from EM lesions in 18 of 21 patients after a minimum of 3 weeks in culture (4). In the current study, of the 21 specimens that were culture positive, 14 (67%) showed growth by 2 weeks of incubation. In eight biopsy cultures there was contamination with other organisms, which precluded identification of *B. burgdorferi*. If these are excluded from analysis, 72% (21 of 29) of the samples obtained from untreated patients resulted in positive cultures. Interestingly, none of the specimens from patients previously treated with antibiotics yielded a positive culture, even though only one or a few doses were received (Table 1). In addition, none of the 10 control biopsy samples produced a positive culture. The high yield of culture obtained in the current study may have been due to careful selection of the reagents used to prepare the BSK medium (6), relatively gentle tissue grinding, strain differences among regional populations of *B. burgdorferi* making isolates from our locale more amenable to in vitro cultivation, or to a more accurate clinical diagnosis of EM.

All of the specimens that were subjected to culture were also tested by the PCR. The number of positive samples obtained with the PCR technique was essentially equivalent to that obtained with culture (59%; 22 of 37). Fifty-nine percent (13 of 22) of the PCR-positive specimens also grew in culture, and 62% (13 of 21) of culture-positive specimens were positive by the PCR. The PCR was successful in detecting *B. burgdorferi* in nine specimens in which cultures were either negative or contaminated. The reason for the failure to detect *B. burgdorferi* by the PCR in eight samples that were culture positive is not clear. Since the skin biopsy tissue was small (2 mm) and then further divided into several parts, it is possible that the number of spirochetes in the portion used for the PCR was below the detection limit of the technique, i.e., the result of a sampling difference in the portions used for culture and the PCR. It is also possible that substances that inhibit the amplification reactions are present in certain skin biopsy samples. It is of interest to note that the sensitivity of *Mycobacterium leprae* detection by the PCR in skin tissue varies greatly depending on the method of sample preparation (9). PCR analysis of the media from the incubating cultures has been carried out; it is worth noting that all eight of the initially PCR-negative, culture-positive samples yielded strong PCR amplification signals on aliquots obtained from 14-day-old cultures (39).

The results obtained from the group of patients with prior antibiotic treatment are noteworthy. None of these specimens produced a positive culture ( $P = 0.002$ ; 0 of 9 versus 21 of 37), and only 2 of 9 specimens were positive by the PCR ( $P = 0.07$ ; 2 of 9 versus 22 of 37) (for comparison of the rate of culture and PCR positivity, respectively, on samples from antibiotic-treated patients versus the corresponding results for samples from untreated patients). This suggests that antibiotic treatment interferes with detection by culture and may also hamper detection by the PCR. The positive PCR results in two of the treated patients may indicate that the

TABLE 2. Culture and PCR results for *B. burgdorferi* compared with patient serologic status for untreated patients with a clinical diagnosis of EM

Serologic status	No. (%) of samples		
	Evaluable <sup>a</sup>	Culture positive	PCR positive
Initially seropositive	16 (46)	10 (71) <sup>b</sup>	11 (69)
Seroconverter <sup>c</sup>	11 (31)	10 (91)	5 (45)
Seronegative <sup>d</sup>	6 (17)	1 (33) <sup>e</sup>	4 (67)

<sup>a</sup> The sum of the numbers of specimens in this column is 33, and not 35 (the total study population), because two patients who were initially seronegative and from whom a second specimen could not be obtained were excluded from the analysis.

<sup>b</sup> Excludes two contaminated specimens.

<sup>c</sup> The initial serum specimen was seronegative, and the second serum specimen was seropositive.

<sup>d</sup> Both first and second serum specimens were seronegative.

<sup>e</sup> Excludes three contaminated samples.

PCR is less prone to this type of interference, but the sample size is too small to allow firm conclusions.

Culture of several specimens was inconclusive because of contamination with other microorganisms, which prevented clear visual identification of *B. burgdorferi*. PCR testing provides a potential advantage in these situations. Five of eight specimens that produced contaminated cultures yielded positive signals by the PCR. Contaminants originating in the skin biopsy sample should not be detected by the PCR assay, since it appears to be highly specific for *B. burgdorferi*, to the exclusion of even closely related *Borrelia* species (Fig. 3), as well as the common bacterial pathogens *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (39).

Ten control specimens from patients undergoing plastic surgery were also tested. One of these samples produced a positive result by the PCR. The problem of false-positive findings in diagnostic applications of the PCR has been described (23), and many of the precautions designed to prevent such results (e.g., use of positive displacement pipets and physical separation of areas for the preparation and analysis of PCR products) were followed in this study. Despite these precautions, an occasional false-positive result does occur. In addition to these control specimens, biopsies from three rashes of unknown etiology and rebiopsy of healthy tissue from 13 culture-positive patients after antibiotic treatment were also examined. None of these samples yielded a positive PCR result. Thus, the specificity of the PCR assay described here is at least 90% (9 of 10) and may be as high as 96% (25 of 26).

By comparison with culture or PCR results, 16 patients (46%) in the nontreated group were seropositive on initial examination. An additional 10 individuals seroconverted within 5 weeks. There was only one case in which the acute-phase serology was positive and both culture and the PCR yielded negative results. Unfortunately, this individual did not return for a follow-up serology test. Thus, the sensitivity of serology was somewhat lower relative to those of culture and the PCR on presentation but comparable when the convalescent serology was considered. The most important advantage of the PCR and culture analyses is that they both provide direct evidence for current infection. This is especially important in an endemic area, where positive serology does not necessarily imply ongoing disease but rather may imply past exposure to *B. burgdorferi*. Serology, however, may be a more appropriate test for patients who have already received antibiotic therapy, since treatment appears to substantially reduce the utility of both culture and the PCR.

Several reports of the use of PCR amplification for the detection and identification of *B. burgdorferi* have appeared recently (11–13, 15, 18, 19, 24, 25, 29). The targets for amplification included chromosomal DNA sequences of unknown function (11, 19, 29), *ospA* (12, 15, 18, 24, 25), and flagellin (13, 24). Several of these studies described the use of PCR amplification for detection of *B. burgdorferi* in human clinical samples (11–13, 15, 19). Goodman et al. were able to detect *B. burgdorferi* in urine samples from four of eight patients with a clinical diagnosis of active late Lyme disease (11). Jaulhac et al. (13) and Keller et al. (15) detected *B. burgdorferi* in the cerebrospinal fluid of patients with neuroborreliosis. Guy and Stanek described detection of *B. burgdorferi* in serum from two of five patients with EM (12). Melchers et al. reported the use of the PCR for the detection of *B. burgdorferi* in skin biopsies from a small number of patients with either EM lesions or acrodermatitis chronica

atrophicans (19). Their sample population included a total of 15 specimens from nine patients (four with EM, five with acrodermatitis chronica atrophicans). Three of the four EM samples were tested by culture, and all were positive; of these, two were also positive by the PCR. The present study encompasses a much larger sample size, including at least 10 control specimens, and therefore represents the most comprehensive study of PCR-based detection of *B. burgdorferi* in patients with early Lyme disease reported to date.

The PCR assay described here is sensitive enough to detect fewer than 10 spirochetes and is highly specific for *B. burgdorferi* (Fig. 3 through 5). The closely related *Borrelia* species tested did not yield a positive PCR signal. In addition, all 10 isolates of *B. burgdorferi* tested in this study, including isolates from North America, Europe, and Russia, yielded an amplified product of the appropriate size. Over 30 additional *B. burgdorferi* isolates have now been examined, and all have produced an identical product on amplification by PCR. This has not been the case with some of the previously reported amplification protocols (25, 29).

Routine laboratory diagnosis of Lyme disease based on culture of the spirochete is less than ideal because of the complexity of culture procedures and the time required. This study indicates that the PCR has an overall sensitivity (59%) for detection of *B. burgdorferi* in skin biopsy specimens from patients with a clinical diagnosis of early Lyme disease similar to that of the culture method (57%). If culture positivity were the standard by which PCR analysis should be assessed, then the sensitivity of the PCR is 62%. The PCR was able to confirm the diagnosis of Lyme disease in approximately 50% of untreated patients who were seronegative at the initial visit and, in contrast to serology, provides direct evidence of active infection. Modifications to the assay designed to increase sensitivity (e.g., improved sample preparation and handling) are currently under investigation. In areas endemic for Lyme disease, diagnosis based on recognition of the characteristic appearance of the EM lesion is the method of choice. However, in cases of atypical lesions or in nonendemic areas, PCR analysis of skin biopsy specimens may provide a more informative technique for diagnosis of early Lyme disease.

#### ACKNOWLEDGMENTS

We thank Robert Nadelman, John Nowakowski, Harold Horowitz, Ulrich Jorde, Marisa Montecalvo, Gilda Forseter, Diane Holmgren, Susan Pond, Donna McKenna, and Karen Buckley for assistance with patients and Roger Salisbury and Jane Petro for providing skin specimens from plastic surgery patients. We thank Alan Barbour and John Anderson for providing isolates of *B. burgdorferi*. We also thank Barbara Johnson, David Dennis, Duane Gubler, and Zvi Loewy for critical reading of the manuscript.

This work was supported in part by the Centers for Disease Control (contract 200-91-0912), the American Lyme Disease Foundation, the New York State Tick-Borne Disease Institute (grant C008374), the National Institutes of Health (Public Health Service grant AR41511), and the New York Medical College Lyme Disease Center.

#### REFERENCES

1. Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.* 57:521–525.
2. Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, G. S. Habicht, T. F. Bast, D. J. Cameron, J. L. Ziegler, A. G. Barbour, W. Burgdorfer, R. Adelman, and R. A. Kaslow. 1983. Spirochetes isolated from the blood of two patients with Lyme disease. *N. Engl. J. Med.* 308:740–742.
3. Berger, B. W. 1989. Cutaneous manifestations of Lyme borreliosis. *Rheum. Dis. Clin. N. Am.* 15:627–634.

4. Berger, B. W., R. C. Johnson, C. Kodner, and L. Coleman. 1992. Cultivation of *Borrelia burgdorferi* from erythema migrans lesions and perilesional skin. *J. Clin. Microbiol.* **30**:359–361.
5. Berger, B. W., M. H. Kaplan, I. R. Rothenberg, and A. G. Barbour. 1985. Isolation and characterization of the Lyme disease spirochete from the skin of patients with erythema chronicum migrans. *J. Am. Acad. Dermatol.* **13**:444–449.
6. Callister, S. M., K. L. Case, W. A. Agger, R. F. Schell, R. C. Johnson, and J. L. E. Ellingson. 1990. Effects of bovine serum albumin on the ability of BSK medium to detect *B. burgdorferi*. *J. Clin. Microbiol.* **28**:363–365.
7. Centers for Disease Control. 1990. Case definitions for public health surveillance. *Morbidity and Mortality Weekly Rep.* **39**(RR-13): 19–21.
8. Dattwyler, R. J., D. J. Volkman, and B. J. Luft. 1989. Immunologic aspects of Lyme borreliosis. *Rev. Infect. Dis.* **11**:s1494–s1498.
9. de Wit, M. Y. L., W. R. Faber, S. R. Krieg, J. T. Douglas, S. B. Lucas, N. Montreewasuwat, S. R. Pattyn, R. Hussain, J. M. Ponnighaus, R. A. Hartskeerl, and P. R. Klatser. 1991. Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissues. *J. Clin. Microbiol.* **29**:906–910.
10. Eisenstein, B. I. 1990. The polymerase chain reaction: a new method of using molecular genetics for medical diagnosis. *N. Engl. J. Med.* **322**:178–183.
11. Goodman, J. L., P. Jurkovich, J. M. Kramber, and R. C. Johnson. 1991. Molecular detection of persistent *Borrelia burgdorferi* in the urine of patients with active Lyme disease. *Infect. Immun.* **59**:269–278.
12. Guy, E. C., and G. Stanek. 1991. Detection of *B. burgdorferi* in patients with Lyme disease by the polymerase chain reaction. *J. Clin. Pathol.* **44**:610–611.
13. Jaulhac, B., P. Nicolini, Y. Piemont, and H. Monteil. 1991. Detection of *Borrelia burgdorferi* in cerebrospinal fluid of patients with Lyme borreliosis. *N. Engl. J. Med.* **324**:1440.
14. Karlsson, M., K. Hovind-Hougen, B. Svenungsson, and G. Stiernstedt. 1990. Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. *J. Clin. Microbiol.* **28**:473–479.
15. Keller, T. L., J. J. Halperin, and M. Whitman. 1992. PCR detection of *Borrelia burgdorferi* DNA in cerebrospinal fluid of Lyme neuroborreliosis patients. *Neurology* **42**:32–42.
16. MacDonald, A. B., B. W. Berger, and T. G. Schwan. 1990. Clinical implications of delayed growth of the Lyme borreliosis spirochete, *Borrelia burgdorferi*. *Acta Trop. Basel* **48**:89–94.
17. Magnarelli, L. A. 1989. Quality of Lyme disease tests. *J. Am. Med. Assoc.* **262**:3464–3465.
18. Malloy, D. C., R. K. Nauman, and H. Paxton. 1990. Detection of *Borrelia burgdorferi* using the polymerase chain reaction. *J. Clin. Microbiol.* **28**:1089–1093.
19. Melchers, W., J. Meis, P. Rosa, E. Claas, L. Nohlmans, R. Koopman, A. Horrevorts, and J. Galama. 1991. Amplification of *Borrelia burgdorferi* DNA in skin biopsies from patients with Lyme disease. *J. Clin. Microbiol.* **29**:2401–2406.
20. Nadelman, R. B., C. S. Pavia, L. A. Magnarelli, and G. P. Wormser. 1990. Isolation of *Borrelia burgdorferi* from the blood of seven patients with Lyme disease. *Am. J. Med.* **88**:21–26.
21. Neubert, U., H. E. Krampitz, and H. Engl. 1986. Microbiological findings in erythema (chronicum) migrans and related disorders. *Zentralbl. Bakteriologie. Mikrobiol. Hyg. Ser. A* **263**:237–252.
22. Pavia, C. S., and C. J. Niederbuhl. 1985. Experimental *Treponema pallidum* infection in inbred guinea pigs: development of lesions and formation of antibodies. *Genitourin. Med.* **61**:75–81.
23. Persing, D. H. 1991. Polymerase chain reaction: trenches to benches. *J. Clin. Microbiol.* **29**:1281–1285.
24. Persing, D. H., S. R. Telford, P. N. Rys, D. E. Dodge, T. J. White, S. E. Malawista, and A. Spielman. 1990. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Ixodes dammini* ticks. *Science* **249**:1420–1423.
25. Persing, D. H., S. R. Telford, A. Spielman, and S. W. Barthold. 1990. Detection of *Borrelia burgdorferi* infection in *Ixodes dammini* ticks by using the polymerase chain reaction. *J. Clin. Microbiol.* **28**:566–572.
26. Peter, J. B. 1991. The polymerase chain reaction: amplifying our options. *Rev. Infect. Dis.* **13**:166–171.
27. Rahn, D. W. 1991. Lyme disease: clinical manifestations, diagnosis and treatment. *Semin. Arthritis Rheum.* **20**:201–218.
28. Rawlings, J. A., P. V. Fournier, and G. J. Tetlow. 1987. Isolation of *Borrelia* spirochetes from patients in Texas. *J. Clin. Microbiol.* **25**:1148–1150.
29. Rosa, P. A., and T. G. Schwan. 1989. A specific and sensitive assay for the Lyme disease spirochete *Borrelia burgdorferi* using the polymerase chain reaction. *J. Infect. Dis.* **160**:1018–1029.
30. Schmidli, J., T. Hunziker, P. Moesli, and U. B. Schaad. 1988. Cultivation of *Borrelia burgdorferi* from joint fluid three months after treatment of facial palsy due to Lyme borreliosis. *J. Infect. Dis.* **158**:905–906.
31. Schwartz, B., M. Goldstein, J. Ribeiro, T. Schulze, and S. Shahied. 1989. Antibody testing in Lyme disease: a comparison of results in four laboratories. *J. Am. Med. Assoc.* **262**:3431–3434.
32. Schwartz, J. J., A. Gazumyan, and I. Schwartz. 1992. rRNA gene organization in the Lyme disease spirochete, *Borrelia burgdorferi*. *J. Bacteriol.* **174**:3757–3765.
33. Snyderman, D. R., D. P. Schenkein, V. P. Berardi, C. C. Lastavica, and K. M. Pariser. 1986. *Borrelia burgdorferi* in joint fluid in chronic Lyme arthritis. *Ann. Intern. Med.* **104**:798–800.
34. Stanek, G., J. Klein, R. Bittner, and D. Glogar. 1990. Isolation of *Borrelia burgdorferi* from the myocardium of a patient with longstanding cardiomyopathy. *N. Engl. J. Med.* **322**:249–252.
35. Steere, A. C. 1989. Lyme disease. *N. Engl. J. Med.* **321**:586–596.
36. Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Kraft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista. 1983. The spirochetal etiology of Lyme disease. *N. Engl. J. Med.* **308**:733–740.
37. Steere, A. C., and S. E. Malawista. 1979. Cases of Lyme disease in the United States: locations correlated with distribution of *Ixodes dammini*. *Ann. Intern. Med.* **91**:730–733.
38. Szczepanski, A., and J. Benach. 1991. Lyme borreliosis: host responses to *Borrelia burgdorferi*. *Microbiol. Rev.* **55**:21–34.
39. Wormser, G. P., S. Bittker, S. L. Bowen, D. Cooper, C. Pavia, and I. Schwartz. Unpublished data.